

questions in the fields of membrane biochemistry and biophysics. It has been suggested that raft domains play a role in signal transduction processes by acting as “signaling platforms”. The three Ras isoforms are posttranslationally modified via lipidation on their C-termini, which is essential for correct functioning and localization at the inner leaflet of the plasma membrane.

By using semisynthetic fully functional lipidated N- and K-Ras proteins, the partitioning of Ras in liquid-disordered (l_d) and liquid-ordered (l_o , i.e., raft-like) subdomains of different artificial and natural membranes was studied by time-lapse atomic force microscopy. The results provide direct evidence that partitioning of Ras occurs preferentially into l_d domains, independent of the lipid anchor system and GDP/GTP-loading [1,2]. Whereas N-Ras proteins bearing at least one farnesyl showed a time dependent diffusion and subsequent clustering in the l_o/l_d phase boundary region, formation of new domains with accumulated protein inside a fluid-like environment was observed for the farnesylated, inactive K-Ras protein. The inserted farnesylated N-Ras proteins are expelled to the interfacial region, probably due to the lack of a particular phase preference, while for the K-Ras protein the strong electrostatic interaction between its positively charged lysines and negatively charged lipids of the membrane seems to control the partitioning behavior. Minimizing the line energy is likely to be one of the key parameters controlling not only the size and dynamic properties of rafts but also of signaling platforms.

References

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Aggregatibacter Actinomycetemcomitans Leukotoxin Disrupts Membranes By Inducing the Formation of An Inverted Hexagonal Lipid Phase

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The leukotoxin (LtxA) secreted by *Aggregatibacter actinomycetemcomitans* is a member of the repeats-in-toxin (RTX) family, and like the other members of the family, is a virulence protein which destroys host cells. LtxA exhibits specificity to human white blood cells, thereby allowing *A. actinomycetemcomitans* to flourish in the human upper aerodigestive tract. Scanning electron micrographs of LtxA-treated human immune cell lines showed that LtxA induces the formation of large pores, which appear to be caused by membrane bending. To understand the molecular mechanism of this interaction, we analyzed the behavior of LtxA-membrane interactions in model membranes. Freeze-fracture transmission electron microscopy revealed the presence of unique structures, such as nanotubules, in multilamellar liposomes treated with LtxA. Formation of these structures indicated that the toxin acts by bending the membrane, possibly by inducing a bilayer-to-nonbilayer transition. This phase transition was quantified using differential scanning calorimetry, and it was found that LtxA is a potent inverted hexagonal (H_{II}) phase promoter. The relationship between H_{II} phase induction and membrane disruption was determined with a calcein leakage assay. LtxA-induced leakage from calcein-encapsulating liposomes composed of lipids with negative curvature, favoring H_{II} phase formation, was significantly enhanced compared to leakage from liposomes composed of lipids with neutral curvature, favoring bilayer formation. Addition of lipids with positive curvature, inhibitors of H_{II} phase formation, or cholesterol sulfate, a bilayer stabilizer, completely eliminated calcein leakage. It appears that LtxA causes membrane disruption by inducing the formation of the H_{II} phase, and its toxic effect is therefore highly dependent on lipid curvature. Supported by NIH DE09517.

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Lateral Pressure Profile and Curvature Frustration in Mechanosensitive Channel Gating

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Experimental evidence of a link between the function and the lipid environment of a membrane protein is increasingly available. While some of the experimental data can be explained by specific lipid-protein interactions, others can only be understood through protein induced perturbations in the membrane shape [1]. In the latter case, the free-energy of a protein state is connected to membrane thickness (hydrophobic mismatch), membrane elasticity and curvature energy [1]. Connection to membrane elasticity and curvature energy can also be formulated using the so-called lateral pressure profile. A conformational change of a membrane protein has to do work against a non-uniform pressure distribution, i.e. lateral pressure profile, inside a membrane [2]. Previously it has been

shown that this work might be significantly larger than thermal energy by assuming simple conformation changes [3].

Here we analyze the work done against the lateral pressure profile in mechanosensitive channel gating using a recently developed method to calculate a full 3D pressure field from molecular dynamics simulations [4]. For this purpose we have simulated closed and open states of a Mechanosensitive channel of large conductance (MscL) embedded in a DOPC bilayer using the MARTINI force field [5]. To analyze the effect of curvature frustration we have also performed simulations with symmetric and asymmetric lipid distributions. The advantage compared to previous analyses [3] is that instead of a simple cone model, we have a more realistic model for different states of a mechanosensitive channel.

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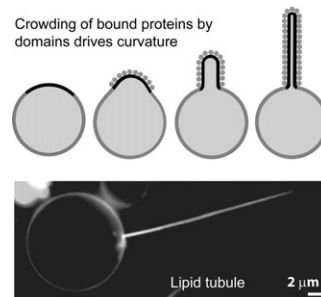
Steric Confinement of Proteins in Lipid Domains Can Drive Membrane Curvature and Tubulation

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Deformation of lipid membranes into curved structures such as buds and tubules is essential to many cellular processes. Lipid micro-domains are thought to co-localize with many curved membrane structures, inspiring ongoing exploration of a variety of roles for domains in membrane bending.

We examined the role of lipid domains in spatial confinement of protein binding and discovered a new mechanism for curvature amplification that relies on global coupling. We formed giant unilamellar vesicles that contained insoluble lipid domains that strongly bound *his*-tagged proteins. We show that protein crowding on domain surfaces creates a protein layer that buckles outward, spontaneously bending the domain into stable, well-defined tubules as more proteins bind. In contrast to previously described bending mechanisms relying on local steric interactions between proteins and lipids (i.e. helix insertion into membranes), this mechanism produces tubules whose dimensions are defined by global parameters: binding energy and domain size. Our results suggest the intriguing possibility that domains can amplify membrane bending and define protrusion length scales by concentrating the steric interactions between the lipid bilayer and proteins. This mechanism may help explain the high curvatures induced by membrane bending proteins.



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Ca²⁺-ATPase: Lipid-Protein Interaction As Observed in Crystals and MD Simulations

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In the quest for grasping the function of complex membrane proteins, it has been realized that the surrounding bilayer may play a regulatory role. Whereas crystal structures in recent years have succeeded in providing detailed images of membrane protein structures, no structural information have appeared clearly showing how the protein is positioned in the membrane. Here we present X-ray data for the sarcoplasmic reticulum Ca²⁺-ATPase (SERCA) in the E2 and E2P states. Structural details have been extracted for the protein and the surrounding bilayer from the same crystal for each of the two configurations. Molecular dynamics (MD) simulations of SERCA incorporated in a bilayer of POPC lipids and detergent (corresponding to crystal conditions), support that the low resolution densities primarily stem from the phosphate (P) groups in the bilayer leaflets.

MD simulations of SERCA in five different single-lipid bilayers show how SERCA, regardless of its extraordinary narrow hydrophobic region, position itself in bilayers of different hydrophobic thickness. Even though membrane proteins are much less compressible than the bilayer, we see both the bilayer and the trans-membrane helix bundle adjust in concert to match their hydrophobic thickness.

